IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application No. 09/803,578

Applicant: Patrick Hwu et al.

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Commissioner for Patents
U.S. Patent and Trademark Office
Randolph Building
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Alexandria, VA 22314

DECLARATION UNDER 37 CFR §1.132 OF PATRICK HWU

Dear Sir:

I, Patrick Hwu, hereby declare the following:

1. I am one of the named inventors of U.S. Patent Application No. 09/803,578 ("the patent application"). I received my Doctor of Medicine degree from the Medical College of Pennsylvania in 1987. I am currently employed by the University of Texas M. D. Anderson Cancer Center, Department of Melanoma Medical Oncology. I am also the first author of P. Hwu et al., *Cancer Res.*, 55: 3369-73 (1995) (hereinafter, "Hwu"), the last author of Kershaw et al. *Nature Biotechnology*, 20: 1221-27 (2002) (hereinafter, "Kershaw") (copy submitted herewith), and an author of Murphy et al. *Cancer Gene Therapy* 14, 499-508 (2007) (copy submitted herewith). I am also an inventor on three U.S. patents.

2. It is my understanding that claim 41 in the patent application reads as follows:

A method of preparing dual specificity lymphocytes comprising:

> (i) contacting lymphocytes in a mixed population of cells with a cell that is allogeneic to one or more lymphocytes, wherein contacting lymphocytes with the allogeneic cell selects and specifically amplifies, from the mixed population of cells, lymphocytes comprising an endogenous receptor that is reactive with the allogeneic cell; and
>
> (ii) transducing the lymphocytes comprising the endogenous receptor reactive with the allogeneic cell with a chimeric receptor gene, said gene encoding a chimeric receptor, which is reactive with a tumor antigen, to produce dual specificity lymphocytes.

3. Hwu teaches transducing murine TILs with chimeric receptor genes, and then co-culturing the transduced TILs with syngeneic cells, i.e., MC38 tumor cells. Hwu teaches that the TIL cultures produced large amounts of mIFN-γ when co-cultured with the MC38 tumor cells.

4. Munz et al. *J. Immunol.*, 162: 25-34 (1999) teaches co-culturing peripheral blood lymphocytes (PBL) with irradiated allogeneic PBL to provide alloreactive T-cells.

5. Tumor antigens are generally weak antigens. "Indeed, tumor antigens are thought to be poor immunogens" (Kershaw, page 122), 1st par.). "...[W]ith the exception of melanoma, tumors have proved to be poorly immunogenic thus far" (Murphy et al., page 505, 1st full par. left column). Accordingly, tumor antigens are not potent enough to generate an immune response to a tumor. Allogeneic cells, on the other hand, are powerful immunogens, but are not capable of generating a specific immune response to a tumor.

6. Successfully generating a potent immune response against a tumor antigen using dual specificity T cells is difficult, and at best, unpredictable. For example, a T-cell that expresses two distinct receptors can exhibit "cross-antagonism," in which the binding of a ligand to one receptor can inhibit a response to the second receptor. One study, for example, used cell lines that expressed two receptors with different specificities and evaluated whether engagement of one receptor by a peptide would result in inhibition of the activation of the T cell line when stimulated by another peptide (Yang et al., *J. Immunol.*, 170: 4532-38 (2003), copy submitted herewith) (hereinafter, "Yang"). Yang showed that an antagonist for one receptor inhibited cell proliferation in response to stimulation of the other receptor (cross-antagonism) in both class I

and class II-restricted, dual-specificity T cells (Yang, page 4536, right column, last par.). Therefore, the success of the dual specificity T cells produced by the claimed method in generating a potent immune response against a tumor antigen would not be predictable.

7. In addition, one of ordinary skill in the art would not have been led to modify the procedure described in Hwu to co-culture the transduced TILs with allogeneic cells. Because the potency of the alloreactive response is so strong, the alloreactive response commandeers the internal "machinery" of the cell (such as, e.g., ZAP 70, and kinases for signal transduction). Therefore, it would not be expected that the introduction into and expression of an exogenous chimeric receptor in the alloreactive cell to produce "dual specificity lymphocytes" having an "endogenous receptor reactive with the allogeneic cell" and a "chimeric receptor, which is reactive with a tumor antigen" would be successful.

8. Moreover, as described in Example 1 of the patent application, the T-cells generated by a method similar to that described in Hwu failed to effectively treat cancer. To summarize, eight patients with advanced ovarian cancer were treated with T-cells transduced in a method similar to that described in Hwu with a chimeric receptor gene (MOv-γ). The cells did not specifically localize at tumor sites. Despite specific *in vitro* reactivity of MOv-PBL against ovarian cancer cells, none of the patients responded to the lymphocyte infusion. Furthermore, the majority of transduced cells were undetectable in circulation between 12-31 days following MOv-PBL infusion (see also, the patent application, Example 1 and Figure 2).

9. In contrast to the poor clinical results obtained with the T-cells transduced in a method similar to that described in Hwu, Example 5 of the patent application demonstrates that T-cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" provided superior experimental results in mice. Dual specificity T-cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" (MOv-γ) provide a T-cell that is specific for both 1) a tumor antigen and 2) an allogeneic cell so that during treatment of a patient, the reactivity of the T-cells against the tumor antigen can be bolstered with immunization with allogeneic cells.

10. To summarize, mice received dual specificity T-cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" (MOv-γ) followed by subcutaneous immunization with allogeneic splenocytes. Mice were later challenged with ovarian cancer tumor cells. As shown in Figure 5 of the patent application, *in vivo* immunization with allogeneic splenocytes from donor mice, combined with administration of dual specificity T cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" (MOv-γ), protected mice much more significantly than T cells alone. Specifically, the combined conditions result in 100% tumor-free mice while mice infused with dual specificity T cells alone resulted in 25% tumor-free mice. These results were also published in Kershaw (see page 1223, left column, Figure 5A).

11. Also in contrast to the poor clinical results obtained with the T-cells of Hwu, Example 6 of the patent application further demonstrates that T-cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" provided superior experimental results to mice.

12. To summarize, mice were injected with tumor cells. Three days later, the mice received either dual specificity T-cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" (MOv-γ), non-dual specific T cells, or no treatment. Mice were immunized with allogeneic splenocytes on days 5, 8, and 11. The dual specificity T cells inhibited the tumor and this effect was augmented by immunization. As shown in Figure 6 of the patent application, mice that were injected with dual specificity T-cells "comprising the endogenous receptor reactive with the allogeneic cell" that were transduced with "a chimeric receptor which is reactive with a tumor antigen" and immunization or boost, resulted in the smallest tumor size throughout the time course of 29 days. These results were also published in Kershaw (see page 1223, left column, Figure 5B).

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that

these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent application or any patent issued thereon.

1/15/09
Date

Patrick Hwu